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METABOLISM OF TESTOSTERONE-4-^{14}C BY HAMSTER SKIN AND FLANK ORGAN*

EDWARD C. GOMEZ, M.D., PH.D., ANTHONY LLEWELLYN, PH.D., AND PHILLIP FROST, M.D.

ABSTRACT

The metabolism of testosterone-4-^{14}C by hamster skin and flank organ was studied; flank organ metabolized more testostrone than an equal weight of adjacent skin. Androstenedione,† androsterone, androstanedione, dihydrotestosterone (DHT), and 3α-androstanediol were identified by chromatography and reverse isotopic dilution as metabolites formed by flank organ. The metabolites of flank organ and general body skin showed identical chromatographic mobilities. Flank organ produced 4 times as much DHT as an equal weight of adjacent skin. The ratio of total 5α-metabolites formed by both tissues was the same as the DHT ratio, indicating that the greater amount of DHT formed is due to greater formation of 5α-metabolites, in general. In contrast, both tissues produced the same amount of 17-ketosteroids from testosterone and had similar DNA contents, suggesting that the observed difference in 5α-reduction was not due to differences in cellularity but to a greater capacity of the flank organ to reduce testosterone. No 3β-hydroxysteroids, the formation of which has been reported in human skin, were found.

The flank organ of the golden hamster, an androgen-sensitive collection of large pilosebaceous units, provides a useful model for the study of sebaceous gland function [1]. This organ has been used for studies of antiandrogenicity [2–4] and studies of dihydrotestosterone (DHT) production [4–6]. The latter studies all confirmed that flank organ reduced the A ring of testosterone to produce DHT but they differed with respect to the other metabolites formed. In view of the large number of metabolites of testosterone produced by human skin [6] and the recent findings suggesting that metabolites other than DHT may play a role in regulating target tissue function, we have examined the in vitro metabolism of testosterone by flank organ and adjacent hamster skin under conditions previously used to study testosterone metabolism in human skin.

In this paper we describe the metabolism of testosterone by both hamster flank organ and adjacent skin, the identification of metabolites produced, and the relative amounts of DHT, 5α-steroids, and 17-ketosteroids formed by flank organ and skin.

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* From the Department of Dermatology, Skin and Cancer Unit, Mount Sinai Medical Center, Miami Beach, Florida 33140.

† The following trivial names and abbreviations are used in this paper: dihydrotestosterone or DHT for 17β-hydroxy-5α-androstan-3-one; androstenedione for androst-4-ene-3,17-dione; androstanedione for 5α-androstane-3,17-dione; epiandrosterone for 3β-hydroxy-5α-androstan-17-one; 3α-androstanediol for 5α-androstane-3α,17β-diol; 3β-androstanediol for 5α-androstane-3β,17β-diol; and etiocholanedione for 5β-androstane-3,17-dione.

MATERIALS AND METHODS

Chemicals. Testosterone-4-^{14}C (sp act, 52.8 μCi/μmole) was purchased from New England Nuclear Corp., and checked for purity by chromatography in a ligroin–propylene glycol system [7]. A methanolic stock solution containing 1.1×10^6 cpm (9.5 nmoles) in each 0.1 ml was prepared and stored at −20°C. Coenzymes were products of Sigma Chemical Co. Reference steroids were purchased from Mann Research Laboratories and Sigma Chemical Company. Reagents were of analytical grade.

Specimens. Golden hamsters were killed by ether anesthesia and the backs shaved with an electric trimmer and razor. The animals were flayed and the subcutaneous fat removed with a scalpel. The flank organ areas were removed with an 8-mm punch biopsy and a specimen of adjacent skin having the same weight as the flank organ areas was taken with the same instrument. The specimens of gland from the same animal were pooled and usually weighed 60–80 mg. Tissues were minced with scissors and kept iced until added to the incubation mixture.

Incubation. Approximately 60–80 mg of minced tissue were added to 3 ml of Krebs–Ringer phosphate buffer (pH 7.4) containing: DPN, 3.3 μmoles; DPNH, 3.3 μmoles; TPN, 3.3 μmoles; glucose-6-phosphate, 12 μmoles; glucose-6-phosphate dehydrogenase, 2 U; penicillin G, 500 U; gentamycin sulfate, 200 μg; and streptomycin, 500 μg. Immediately prior to incubation, 0.1 ml of stock solution of radioactive substrate was added. Incubation was carried out at 37°C for 5 hr in a Dubnoff shaking incubator.

Extraction. The incubation was terminated by adding 100 μg each of nonradioactive testosterone, dihydrotestosterone, epiandrosterone, androstenedione, androsterone, and androstanedione, followed by 15 ml of a mixture of methanol:dichloromethane (2:1). The tissue and small amounts of precipitate were removed by filtration and washed with the same solvents. The filtrate and washings were combined and concentrated under vacuum and extracted as previously described [8].

Chromatography. The extracted residue was analyzed by chromatography in the following systems:

System PG: the ligroin-propylene glycol system of Savard [7].

System TPG: a toluene-propylene glycol system similar to system PG except for using toluene, instead of ligroin, as the mobile phase.

System TLC-CM-4: a thin-layer chromatography system using silica gel H and a solvent system consisting of chloroform:methanol (94:4). The chloroform was stabilized by the addition of ethanol immediately after distillation to give a final concentration of 0.75% ethanol. The details of this chromatography system were described previously [9].

The distribution of radioactivity in the chromatograms was monitored with a Vanguard Autoscanner 880 with a Nuclear Chicago integrator.

Isotopic dilutions. The different radioactive areas were eluted from chromatograms with methanol. In addition to chromatographic behavior, the technique of reverse isotopic dilution was used to further establish the identity of metabolites. Details of the methods used have been published previously [8, 10]. Specific activity of the crystals was determined by weighing well-formed crystals in a Cahn electrobalance, dissolving in scintillation fluid, and measuring the radioactivity in a Beckman Model LS-100 liquid scintillation counter with an external standard. Isotopic dilutions were continued until three consecutive assays of the crystals indicated a constant specific activity.

DNA determinations. The flank organs and an equal weight of adjacent skin were excised from 7 male hamsters. After weighing, 14 flank organs were pooled as were the 7 skin specimens. The DNA of both flank organs and skin was then extracted and measured by the method of Burton [11], as modified by Davis [12].

RESULTS

Testosterone-4-^{14}C was incubated separately with the excised flank organs of a hamster and with an equal weight of the adjacent skin from the same animal. After extraction, the residue was chromatographed in system PG for 12 hr. The run-off from these chromatograms was collected and run in the same chromatographic system for 4 hr. As shown in Figure 1, both flank organ and skin produced metabolites with similar chromatographic patterns. The initial chromatograms in system PG showed four radioactive areas corresponding to peaks II, III, IV, and V. Peak IV, which was the largest peak, corresponded to the position of the testosterone carrier. In both chromatograms, peak II corresponded to the position of the carriers, androsterone and androstenedione; peak III, to the mobility of DHT. When the run-off from these chromatograms was analyzed by chromatography in system PG for a shorter time, two additional peaks were found; peak I corresponded to the position of androstanedione, and peak 0 was a nonpolar material which moved with the solvent front and did not correspond to the mobility of any reference steroid.

The chromatograms were cut and each of the radioactive areas was eluted with methanol. On chromatography in system TLC-CM-4, peak I showed the mobility of androstanedione and peak III that of DHT. Also in this system, peak II was resolved into two components, peak IIa, with the mobility of androstenedione, and peak IIb, with the mobility of androsterone. It was noted that the incubation with flank organ produced more peak IIb than IIa, whereas, with skin, the reverse situation existed. Peaks IV and V were combined and chromatographed in system TPG in which similar chromatographic patterns were obtained with both flank organ and skin (Fig. 2). Most of the radioactivity was associated with the testosterone carrier (peak IVa). Just behind the testosterone carrier was a second radioactive area (peak IVb) with the mobility of 3α-androstanediol, which moves between peak IVa and peak V. Peaks IVa and IVb were eluted from the chromatogram and chromatographed in system TLC-CM-4 in which they had the mobility of testosterone and 3α-androstanediol, respectively.

Isotopic Dilutions

The chromatographic identification of the metabolites was confirmed by reverse isotopic dilution. As shown in Table I, the radioactive material from peak I was diluted with androstanedione to

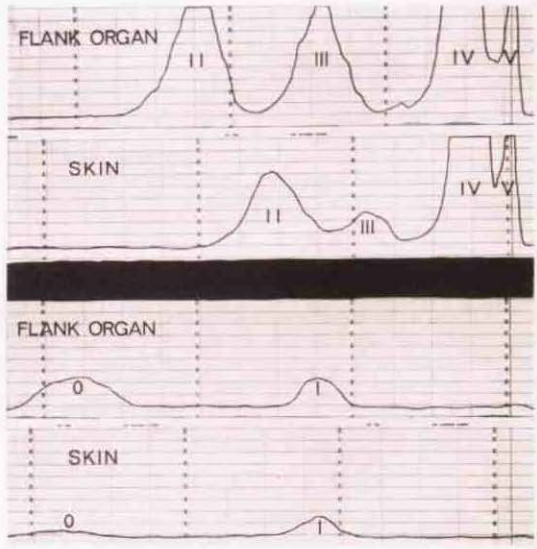

FIG. 1: Initial chromatography in system PG. The top tracings show the distribution of radioactivity obtained after chromatography in system PG for 12 hr. The lower tracing shows the distribution obtained when the run-off from the 12-hr chromatograms was run in the same system for 4 hr. The radioactive areas corresponded in mobility to the following carrier steroids: peak I, androstanedione; peak II, androstenedione and androsterone; peak III, DHT and epiandrosterone; peak IV, testosterone. (Migration rates were compared to carriers on the same strip so that differences in rate of development, as in the top tracings, do not influence identification by relative mobility).

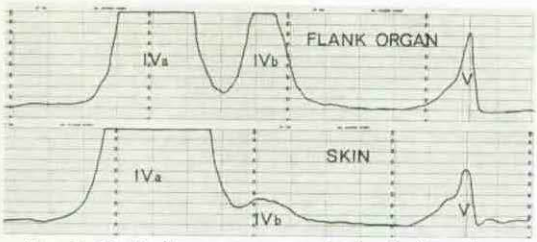

FIG. 2: Radiochromatograms obtained when the areas corresponding to peaks IV and V of the chromatograms in Figure 1 were eluted and chromatographed for 6 hr in system TPG. Peak IVa corresponded to the mobility of the testosterone carrier and peak IVb to the mobility of a 3α-androstanediol standard. No radioactivity was associated with the 3β-androstanediol standard which moved between peak IVb and peak V at the origin.

TABLE I

Identification of metabolites from flank organ

Peak	Standard	Calc. sp act[a]	# of cryst.	Sp act
I	5α-Androstanedione	185	2	180
			4	179
			6	180
IIa	Androstenedione	610	2	604
			4	591
			6	597
IIb	Androsterone	274	1	254
			2	249
			3	268
III	Dihydrotestosterone	243	2	237
			4	227
			6	225
IV[b]	Testosterone	1188	2	1103
			4	1092
			6	1062
IVb	Androstanediol	776	1	700
			2	664
			3	670

[a] A known amount of radioactivity eluted from the areas of the indicated peak was mixed with a known weight of nonradioactive steroid standard and the specific activity of the mixture calculated.

[b] The material used for this isotopic dilution was peak IV from the initial chromatography in system PG and thus contained both peak IVa and IVb.

give a calculated specific activity of 185 dpm/μmole. After six crystallizations, the specific activity was unchanged. Because of the difficulty in separating androstanedione chromatographically from its 5β-isomer, etiocholandione, an aliquot of radioactivity from peak I was also subjected to reverse isotopic dilution with etiocholanedione. After six crystallizations, the specific activity had decreased from the calculated value of 264 dpm/μmole to 3 dpm/μmole. These data suggest that, as in human skin, the reduction of the double bond of testosterone is stereospecific, producing the 5α-isomer, androstanedione. Similarly, the identification of androstenedione, androstanedione, DHT, and 3α-androstanediol were confirmed by isotopic dilution. Peak IV was also examined by isotopic dilution with testosterone.

Because of the lesser amounts of metabolites obtained with hamster skin as compared to flank organ, the identification of the metabolites by isotopic dilutions was not carried out, except in the case of androstenedione, which is the major metabolite of hamster skin. An aliquot of the radioactive material from peak IIa obtained by incubation with hamster skin was mixed with cold androstenedione to yield a calculated specific activity of 710 dpm/μmole. After two crystallizations the actual specific activity was 700 dpm/μmole, and after six crystallizations, 705 dpm/μmole. These data confirm the identification of the material in peak IIa produced by hamster skin as androstenedione.

Comparison of Metabolism by Hamster Flank Organ and Adjacent Skin

To compare the production of DHT and other metabolites by hamster flank organ and adjacent skin, duplicate incubations were carried out using two male hamsters and incubating simultaneously the pair of flank organs obtained from each hamster and an equal weight of the adjacent skin from each animal. The amount of radioactivity in each of the various metabolites was determined, as shown in Table II. Hamster skin metabolized approximately 13% of the testosterone, whereas an equal weight of flank organ metabolized approximately 33%. Hamster skin produced less of each of the various metabolites with the exception of androstenedione (peak IIa).

As shown in Figure 3, which is derived from the

TABLE II

Percent of radioactivity in each peak

Tissue	Peak[a]							
	0	I	IIa	IIb	III	IVa	IVb	V
Flank organ	3.2	2.6	1.2	2.0	8.6	66.9	12.3	3.2
Skin	0.6	0.8	3.9	0.6	1.8	87.1	2.8	2.4

[a] Key to metabolites;

0 Unidentified
I 5α-Androstane-3,17-dione
IIa Androst-4-ene-3,17-dione
IIb Androsterone
III Dihydrotestosterone
IVa Testosterone (substrate)
IVb 5α-Androstane-3α,17β-diol
V Unidentified

Values indicated are the average of duplicate experiments.

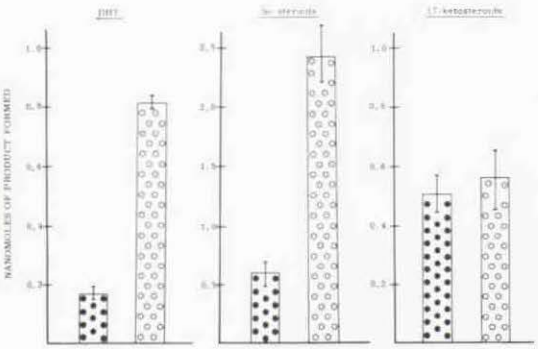

FIG. 3: Metabolites produced by flank organ (open circles) and skin (closed circles). *Left:* DHT production. *Middle:* total 5α-steroids produced. *Right:* total 17-ketosteroids produced. The brackets indicate the values obtained in duplicate experiments.

data in Table II, the flank organ produced about 4 times as much DHT as an equal weight of the adjacent skin. To determine whether these data represented a specific increase in DHT formation or simply an increase in the reduction of the Δ^4-double bond, all of the 5α-metabolites were totaled and, as shown in the middle graph of Figure 3, flank organ produced more total 5α-steroids than did the adjacent skin. In addition, the ratio of total 5α-steroids formed in flank organ to that formed in skin was approximately 4 to 1, which is approximately the same ratio observed for DHT. These data suggest that the greater production of DHT is due to an increase in Δ^4-double bond reduction rather than to a specific accumulation of DHT.

To investigate the possibility that the greater 5α-reduction observed with flank organ might be due to an increased cellular content within the flank organ, the data were examined for another independent steroid transformation, the oxidation of the 17β-hydroxyl group to yield 17-ketosteroids. By totaling the 17-ketosteroids produced, it was found that there was little difference between skin and flank organ (Fig. 3).

DNA Content of Hamster Flank Organ and Skin

To further compare the cellular content of flank organ and skin, the DNA content of each was determined as described under *Materials and Methods*. The flank organ contained 2.9 μg of DNA/mg of flank organ, while skin contained 3.2 μg of DNA/mg of skin. There does not, therefore, appear to be a significant difference in the cell content of flank organ and skin and, if corrected for the values obtained, the difference in 5α-reduction observed on a weight basis would be augmented rather than decreased.

DISCUSSION

The association of DHT production with target tissues of androgens has been described by Bruchovsky and Wilson [13] and lends support to the concept of DHT as the "tissue active" form of testosterone in certain organs. Skin is a complex mixture of cell types, only a few of which are androgen-responsive end organs. We reported that flank organ, a region rich in sebaceous units, produced more DHT than adjacent skin [5]. Takayasu and Adachi [6] demonstrated DHT production by sebaceous glands mechanically isolated from flank organ, demonstrating that this reaction can occur within the target tissue itself, as had been described for prostate. The present data indicate that the greater production of DHT by flank organ is a reflection of the generally greater production of 5α-steroids and is not limited to DHT production. Also, this increased capacity of flank organ for 5α-reduction does not appear to result from greater cellularity since there is no significant difference in the DNA content of the flank organ and adjacent skin or in the amount of 17-keto metabolites produced by tissue from these two sites. These facts suggest that, although areas devoid of sebaceous glands (palms and soles) can convert testosterone to DHT [14], areas rich in well-developed sebaceous glands have greater capacity for this specific reaction. This is in accordance with the finding of Sansone and Reisner [15] with human skin from various sites.

Studies of cutaneous testosterone metabolism have centered around DHT production and have utilized homogenates to which only TPNH or a TPNH generating system was added as cofactor. The major product under such conditions has been DHT. When minced tissue is incubated or when DPN and DPNH are added, a variety of 3-hydroxy and 17-keto steroids are produced by human skin [8] and by hamster skin and flank organ. Lasnitzki et al‡ have demonstrated that, whereas DHT can cause hyperplasia in prostatic organ cultures, a second metabolite of testosterone, 3β-androstanediol, is more potent in stimulating secretion. Recently, Ebling [16] demonstrated that 3β-androstanediol significantly increased sebum production in rats. Production of 3β-androstanediol from testosterone has recently been reported in human skin [17], but the present data indicate that this metabolite is not produced by hamster skin or flank organ. Since such subtle differences in metabolism may be significant, data obtained with such model systems must be interpreted cautiously as regards human sebaceous gland function.

‡ Lasnitzki I, Baulieu EE, Robel P: Abstract #18. Presented at the 51st Meeting of the Endocrine Society, 1969.

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